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(71) Applicant *(for all designated States except US)*: AMGEN INC. [US/US]; One Amgen Center Drive, Thousand Oaks, CA 91320–1799 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: MAGAL, Ella [IL/US]; 3022 Windrift Court, Thousand Oaks, CA 91360 (US).

(74) Agents: ODRE, Steven, M. et al.; Amgen, Inc., One Amgen Center Drive, Thousand Oaks, CA 91320–1799 (US).

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(54) Title: METHOD FOR PREVENTING AND TREATING HEARING LOSS USING SENSORINEUROTROPHIC COMPOUNDS

amount of a sensorineurotrophic compound. According to one aspect of the invention, methods are provided for treating damaged hair cells and auditory neurons by administering a therapeutically effective amount of a sensorineurotrophic compound by means of a pharmaceutical composition.

The present invention is based on the discovery that a sensorineurotrophic compound protects hair cells from ototoxin-induced cell death in explant cultures of rat's cochlea and in an animal model (guinea pig) of deafness. It is contemplated that administration of exogenous sensorineurotrophic compound will protect hair cells and spiral ganglion neurons from traumatic damage, for example damage caused by noise trauma, acute or chronic treatment with cisplatin and aminoglycoside antibiotics or from damage resulting from a lack of neurotrophic factors resulting from interruption of transport of the factors from the axon to the cell body. Such treatment is expected to allow hair cells and/or auditory neurons to tolerate intermittent insults from either environmental noise trauma or treatment with ototoxins, and to slow down, prevent or reverse the progressive degeneration of the auditory neurons and hair cells which is responsible for hearing loss in pathological conditions such as presbycusis (age-related hearing loss), inherited sensorineural degeneration, and post-idiopathic hearing losses and to preserve the functional integrity of the inner ear. Such treatment will also support the auditory neurons for better and longer performance of cochlear implants.

According to the invention, the sensori-neurotrophic compound may be administered systemically at a dose ranging from about 1 to about 10 mg/kg/day or into the middle ear at a dose ranging from about 1 ng/ear/day to

about 10 ng/ear/day, typically at a dose of about 1 µg ear/day to about 10 µg/ear/day, and usually at a dose of about 5 µg/ear/day to about 20 µg/ear/day. The sensorineurotrophic compound may be administered directly into the inner ear in cases where invasion of the inner ear has already occurred such as in surgical procedures for inserting a cochlear implant or other surgeries of the inner ear. In such cases, a smaller amount of sensorineurotrophic compound may be administered, for example, from about 0.1 ng/ear to about 1 ng/ear in a single injection or in multiple injections. The sensorineurotrophic compound can be prepared and administered in the form of ear-drops which will penetrate the tympanic membrane. It is further contemplated that the sensorineurotrophic compound may be administered with an effective amount of a second therapeutic agent for the treatment of auditory neuron degeneration, including GDNF, BDNF and NT-3 as well as other factors or drugs used currently or in the future for the treatment of various inner and middle ear pathologies. A variety of pharmaceutical formulations and different delivery techniques are described in further detail below.

C. Sensorineurotrophic Compound Pharmaceutical Compositions

Sensorineurotrophic compound pharmaceutical compositions typically include a therapeutically effective amount of a sensorineurotrophic compound described herein in admixture with one or more pharmaceutically and physiologically acceptable formulation materials. Suitable formulation materials include, but are not limited to, antioxidants, preservatives, coloring, flavoring and diluting agents,

emulsifying agents, suspending agents, solvents, fillers, bulking agents, buffers, delivery vehicles, diluents, excipients and/or pharmaceutical adjuvants. For example, a suitable vehicle may be water for injection, physiological saline solution, or artificial perilymph, possibly supplemented with other materials common in compositions for parenteral administration. Neutral buffered saline or saline mixed with serum albumin are further exemplary vehicles.

The primary solvent in a vehicle may be either aqueous or non-aqueous in nature. In addition, the vehicle may contain other pharmaceutically-acceptable excipients for modifying, modulating or maintaining the pH, osmolarity, viscosity, clarity, color, sterility, stability, rate of dissolution, or odor of the formulation. Similarly, the vehicle may contain still other pharmaceutically-acceptable excipients for modifying or maintaining the rate of release of the therapeutic product(s), or for promoting the absorption or penetration of the therapeutic product(s) across the tympanic membrane. Such excipients are those substances usually and customarily employed to formulate dosages for middle-ear administration in either unit dose or multi-dose form.

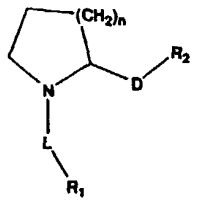
Once the therapeutic composition has been formulated, it may be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or dehydrated or lyophilized powder. Such formulations may be stored either in a ready to use form or in a form, e.g., lyophilized, requiring reconstitution prior to administration.

The optimal pharmaceutical formulations will be determined by one skilled in the art depending upon considerations such as the route of administration and

desired dosage. See, for example, "Remington's Pharmaceutical Sciences", 18th ed. (1990, Mack Publishing Co., Easton, PA 18042), pp. 1435-1712, the disclosure of which is hereby incorporated by reference. Such formulations may influence the physical state, stability, rate of in vivo release, and rate of in vivo clearance of the present therapeutic agents of the invention.

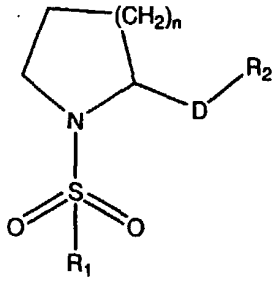
Other effective administration forms, such as middle-ear slow-release formulations, inhalant mists, or orally active formulations are also envisioned. For example, in a sustained release formulation, the sensorineurotrophic compound may be bound to or incorporated into particulate preparations of polymeric compounds (such as polylactic acid, polyglycolic acid, etc.) or liposomes. Hylauronic acid may also be used, and this may have the effect of promoting sustained duration in the circulation. The sensorineuro-trophic compound pharmaceutical composition also may be formulated for middle-ear administration, e.g., by tympanic membrane infusion or injection, and may also include slow-release or sustained circulation formulations. Such middle-ear administered therapeutic compositions are typically in the form of a pyrogen-free, middle-ear acceptable aqueous solution comprising the sensorineurotrophic compound in a pharmaceutically acceptable vehicle. One preferred vehicle is sterile distilled water.

Certain formulations containing a sensori-neurotrophic compound may be administered orally. A sensorineurotrophic compound which is administered in this fashion may be encapsulated and may be formulated with or without those carriers customarily used in the compounding of solid dosage forms. The capsule may be designed to release the active portion of the formulation



No.	n	D	R_2	L	R_1
713	1	CH_2	OH	1,2-dioxoethyl	benzyl
714	1	bond	-CN	1,2-dioxoethyl	1,1-dimethylpropyl
715	1	bond	tetrazole	1,2-dioxoethyl	1,1-dimethylpropyl
716	2	bond	$CONH_2$	1,2-dioxoethyl	1,1-dimethylpropyl
717	1	bond	COOH	1,2-dioxoethyl	1,1-dimethylpropyl
718	2	bond	COOH	1,2-dioxoethyl	1,1-dimethylpropyl

A another preferred embodiment of the invention is the use for the treatment or prevention of sensorineural hearing loss with a compound of the formula (LXVII):



(LXVII)

in which:

n is 1-3;

R_1 is selected from the group consisting of hydrogen, C_1-C_9 straight or branched chain alkyl, C_2-C_9 straight or branched chain alkenyl, aryl, heteroaryl, carbocycle, or heterocycle;

D is a bond, or a C_1-C_{10} straight or branched chain alkyl, C_2-C_{10} alkenyl or C_2-C_{10} alkynyl;

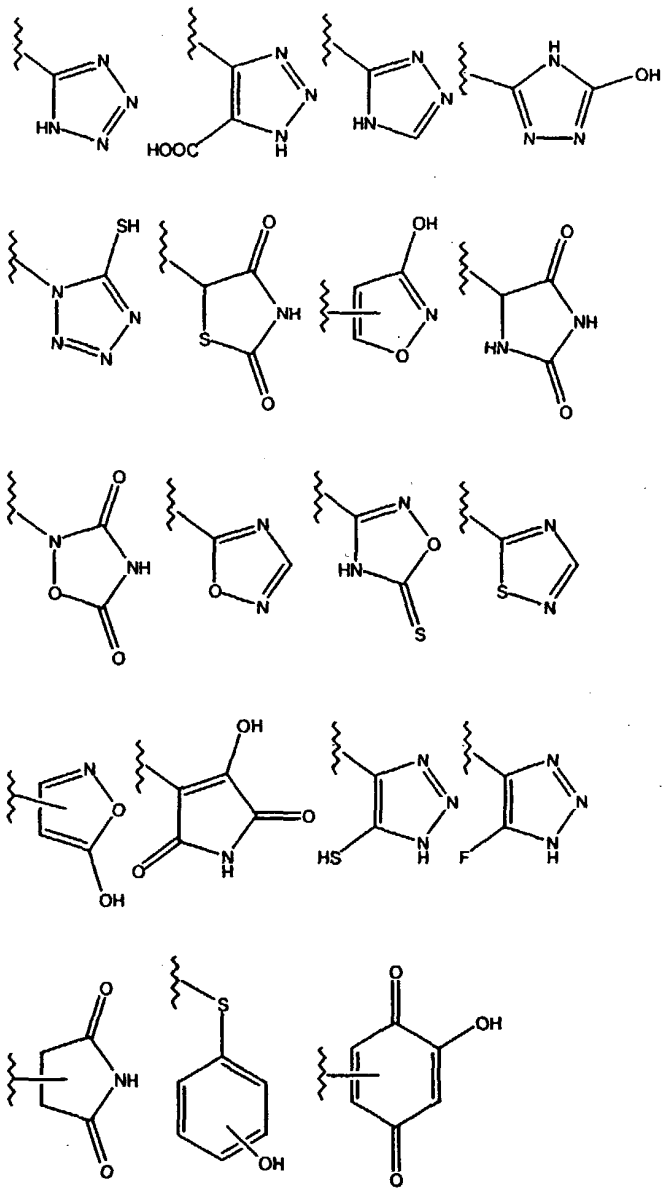
R_2 is a carboxylic acid or a carboxylic acid isostere;

wherein said alkyl, alkenyl, alkynyl, aryl, heteroaryl, carbocycle, heterocycle, or carboxylic acid isostere is optionally substituted with one or more

substituents selected from R^3, where
R^3 is hydrogen, hydroxy, halo, , halo-(C_1-C_6)-alkoxy, thiocarbonyl, (C_1-C_6)-alkoxy, (C_2-C_6)-alkenyloxy, (C_1-C_6)-alkylaryloxy, aryloxy, aryl-(C_1-C_6)-alkyloxy, cyano, nitro, imino, (C_1-C_6)-alkylamino, amino-(C_1-C_6)-alkyl, sulfhydryl, thio-(C_1-C_6)alkyl, (C_1-C_6)-alkylthio, sulfonyl, C_1-C_6 straight or branched chain alkyl, C_2-C_6 straight or branched chain alkenyl or alkynyl, aryl, heteroaryl, carbocycle, heterocycle, or CO_2R^4 where R^4 is hydrogen or C_1-C_9 straight or branched chain alkyl or alkenyl;
or a pharmaceutically acceptable salt, ester or solvate thereof;

A preferred embodiment of this invention is the use of a compound in which R_2 is a carbocycle or heterocycle containing any combination of CH_2, O, S, or N in any chemically stable oxidation state, where any of the atoms of said ring structure are optionally substituted in one or more positions with R^3.

Especially preferred embodiments of this aspect of the invention are the use of those compounds in which R_2 is selected from the group below:



in which the atoms of said ring structure may be optionally substituted at one or more positions with R^3.

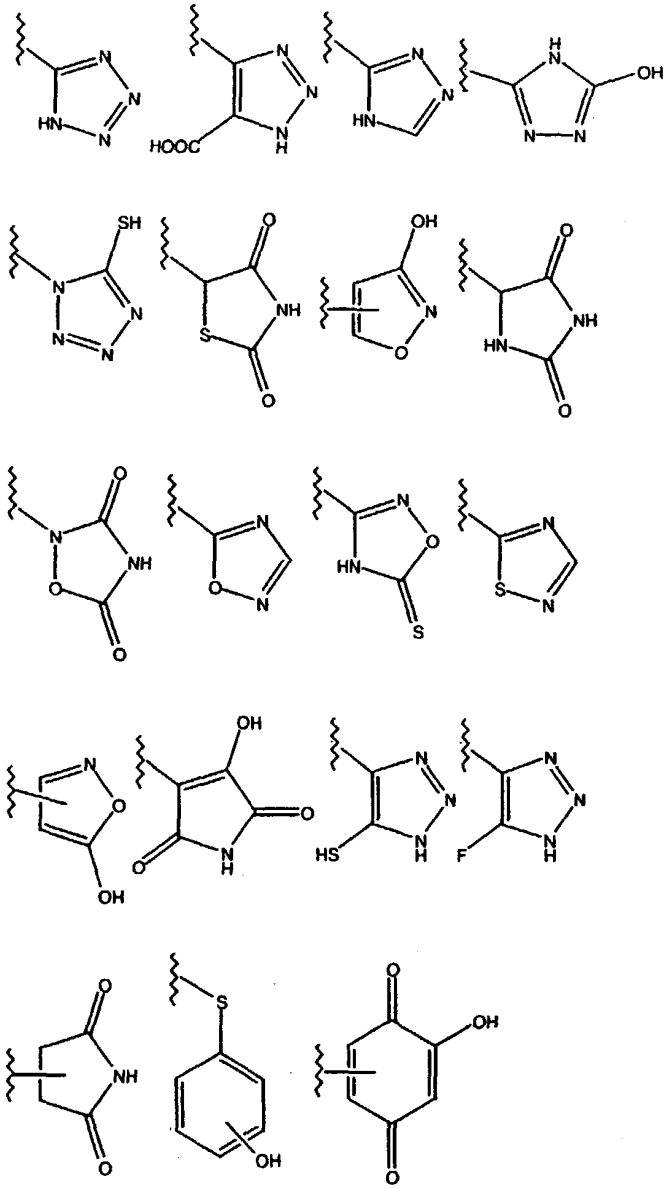
Another preferred embodiment of this invention is where R_2 is selected from the group consisting of -COOH, $-SO_3H$, $-SO_2HNR^3$, $-PO_2(R^3)_2$, -CN, $-PO_3(R^3)_2$, $-OR^3$, $-SR^3$, $-NHCOR^3$, $-N(R^3)_2$, $-CON(R^3)_2$, $-CONH(O)R^3$, $-CONHNHSO_2R^3$, $-COHNSO_2R^3$, and $-CONR^3CN$.

Preferred embodiments of this invention are the following compounds: (2S)-1-(phenylmethyl)sulfonyl-2-hydroxymethyl pyrrolidine; (2S)-1-(phenylmethyl)-sulfonyl-2-pyrrolidinetetrazole; (2S)-1-(phenyl-methyl)-sulfonyl-2-pyrrolidine carbonitrile; and compounds 719-821.

"Isosteres" are different compounds that have different molecular formulae but exhibit the same or similar properties. For example, tetrazole is an isostere of carboxylic acid because it mimics the properties of carboxylic acid even though they both have very different molecular formulae. Tetrazole is one of many possible isosteric replacements for carboxylic acid. Other carboxylic acid isosteres contemplated by the present invention include $-COOH$, $-SO_3H$, $-SO_2HNR^3$, $-PO_2(R^3)_2$, $-CN$, $-PO_3(R^3)_2$, $-OR^3$, $-SR^3$, $-NHCOR^3$, $-N(R^3)_2$, $-CON(R^3)_2$, $-CONH(O)R^3$, $-CONHNHSO_2R^3$, $-COHNSO_2R^3$, and $-CONR^3CN$, wherein R^3 is hydrogen, hydroxy, halo, halo-C_1-C_6-alkyl, thiocarbonyl, C_1-C_6-alkoxy, C_2-C_6-alkenoxy, C_1-C_6-alkylaryloxy, aryloxy, aryl- C_1-C_6-alkyloxy, cyano, nitro, imino, C_1-C_6-alkylamino, amino-C_1-C_6-alkyl, sulfhydryl, thio- C_1-C_6-alkyl, C_1-C_6-alkylthio, sulfonyl, C_1-C_6 straight or branched chain alkyl, C_2-C_6 straight or branched chain alkenyl or alkynyl, aryl, heteroaryl, carbocycle, heterocycle, and CO_2R^4 where R^4 is hydrogen or C_1-C_9 straight or branched chain alkyl or alkenyl.

In addition, carboxylic acid isosteres can include 5-7 membered carbocycles or heterocycles containing any combination of CH_2, O, S, or N in any chemically stable oxidation state, where any of the atoms of said ring structure are optionally substituted in one or more positions. The following structures are non-limiting examples of preferred carbocyclic and heterocyclic

isosteres contemplated by this aspect of the invention.



where the atoms of said ring structure may be optionally substituted at one or more positions with R^3. The present invention contemplates that when chemical substituents are added to a carboxylic isostere then the inventive compound retains the properties of a carboxylic isostere. The present invention contemplates that when a carboxylic isostere is optionally substituted with one or

more moieties selected from R^3, then the substitution can not eliminate the carboxylic acid isosteric properties of the inventive compound. The present invention contemplates that the placement of one or more R^3 substituents upon a carbocyclic or heterocyclic carboxylic acid isostere shall not be at an atom(s) which maintains or is integral to the carboxylic acid isosteric properties of the inventive compound if such a substituent(s) would destroy the carboxylic acid isosteric properties of the inventive compound.

Other carboxylic acid isosteres not specifically exemplified or described in this specification are also contemplated by the present invention.

A compound of the present invention, especially formula LXVII, wherein n is 1, D is a bond, R_1 is phenylmethyl, and R_2 is -CN, is named (2S)-1-(phenylmethyl) sulfonyl-2-pyrrolidine carbonitrile.

Specific embodiments of the inventive compounds are presented in Table XLVIII. The present invention contemplates employing the compounds of Table XLVIII, below, for use in compositions and methods of the invention.

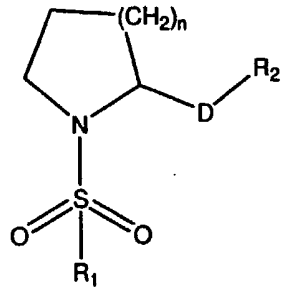
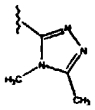
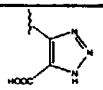
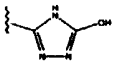
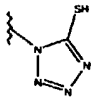
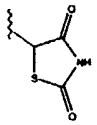
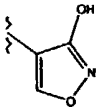
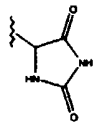
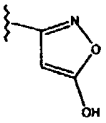
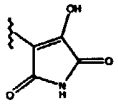
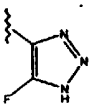
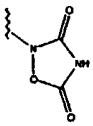
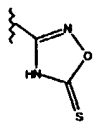


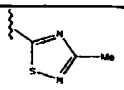
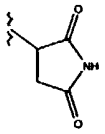
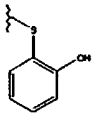
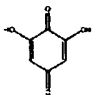
TABLE XLVIII

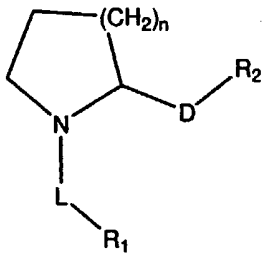
No.	n	D	R_2	R_1
719	1	bond	COOH	Benzyl
720	1	bond	COOH	a-MethylBenzyl
721	1	bond	COOH	4-MethylBenzyl

No.	n	D	R_2	R_1
722	1	bond	Tetrazole	Benzyl
723	1	bond	SO_3H	a-MethylBenzyl
724	1	CH_2	COOH	4-MethylBenzyl
725	1	bond	SO_2HNMe	Benzyl
726	1	bond	CN	a-MethylBenzyl
727	1	bond	PO_3H_2	4-MethylBenzyl
728	2	bond	COOH	Benzyl
729	2	bond	COOH	a-MethylBenzyl
730	2	bond	COOH	4-MethylBenzyl
731	2	bond	COOH	3,4,5-trimethoxy-phenyl
732	2	bond	COOH	Cyclohexyl
733	2	bond	PO_2HEt	i-propyl
734	2	bond	$PO_3HPropyl$	ethyl
735	2	bond	$PO_3(Et)_2$	Methyl
736	2	bond	OMe	tert-butyl
737	2	bond	OEt	n-pentyl
738	2	bond	OPropyl	n-hexyl
739	1	bond	OButyl	Cyclohexyl
740	1	bond	OPentyl	cyclopentyl
741	1	bond	OHexyl	n-heptyl
742	1	bond	SMe	n-octyl
743	1	bond	SEt	n-nonyl
744	2	bond	SPropyl	2-indolyl
745	2	bond	SButyl	2-furyl
746	2	bond	NHCOMe	2-thiazolyl
747	2	bond	NHCOEt	2-thienyl
748	1	CH_2	$N(Me)_2$	2-pyridyl
749	1	$(CH_2)_2$	N(Me)Et	benzyl
750	1	$(CH_2)_3$	$CON(Me)_2$	benzyl
751	1	$(CH_2)_4$	CONHMe	benzyl
752	1	$(CH_2)_5$	CONHEt	benzyl
753	1	$(CH_2)_6$	CONHPropyl	1,1-dimethylpropyl
754	1	bond	CONH(O)Me	Benzyl
755	1	bond	CONH(O)Et	a-Methylphenyl
756	1	bond	CONH(O)Propyl	4-Methylphenyl
757	2	bond	COOH	Benzyl
758	2	bond	COOH	a-Methylphenyl
759	2	bond	COOH	4-Methylphenyl
760	1	CH_2	COOH	benzyl

No.	n	D	R_2	R_1
761	1	$(CH_2)_2$	COOH	benzyl
762	1	$(CH_2)_3$	COOH	benzyl
763	1	$(CH_2)_4$	COOH	benzyl
764	1	$(CH_2)_5$	COOH	benzyl
765	1	$(CH_2)_6$	COOH	benzyl
766	1	$(CH_2)_7$	COOH	benzyl
767	1	$(CH_2)_8$	COOH	benzyl
768	1	$(CH_2)_9$	COOH	benzyl
769	1	$(CH_2)_{10}$	COOH	benzyl
770	1	C_2H_2	COOH	benzyl
771	1	2-hydroxyethyl	COOH	benzyl
772	1	2-butylene	COOH	benzyl
773	1	i-Propyl	COOH	benzyl
774	1	tert-Butyl	COOH	benzyl
775	1	2-nitrohexyl	COOH	benzyl
776	3	$(CH_2)_2$	CN	benzyl
777	1	$(CH_2)_3$	CN	benzyl
778	3	bond	$CONHNHSO_2Me$	Benzyl
779	3	bond	$CONHNHSO_2Et$	a-Methylphenyl
780	3	bond	$CONHSO_2Me$	4-Methylphenyl
781	2	bond	$CONHNHSO_2Et$	Phenyl
782	2	bond	CON(Me)CN	a-Methylphenyl
783	2	bond	CON(Et)CN	4-Methylphenyl
784	1	$(CH_2)_2$	COOH	methyl
785	1	$(CH_2)_3$	COOH	ethyl
786	1	$(CH_2)_4$	COOH	n-propyl
787	1	$(CH_2)_5$	COOH	t-butyl
788	1	$(CH_2)_6$	COOH	Pentyl
789	1	$(CH_2)_7$	COOH	Hexyl
790	1	$(CH_2)_8$	COOH	Heptyl
791	1	$(CH_2)_9$	COOH	Octyl
792	1	$(CH_2)_{10}$	COOH	Nonyl
793	1	C_2H_2	COOH	Cyclohexyl
794	1	bond		benzyl
795	1	bond		benzyl

No.	n	D	R_2	R_3
796	1	bond		benzyl
797	1	bond		benzyl
798	1	bond		benzyl
799	1	bond		benzyl
800	1	bond		benzyl
801	1	bond		benzyl
802	1	bond		benzyl
803	1	bond		benzyl
804	1	bond		benzyl
805	1	bond		benzyl
806	1	bond		benzyl
807	1	bond		benzyl
808	1	bond		benzyl

No.	n	D	R_2	R_1
809	1	bond		benzyl
810	1	bond		benzyl
811	1	bond		benzyl
812	1	bond		benzyl
813	1	bond	CH_2OH	benzyl
814	1	bond	$CONH_2$	benzyl
815	1	bond	CN	benzyl



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No.	n	D	R_2	L	R_1
816	1	CH_2	OH	1,2-dioxoethyl	benzyl
817	1	bond	-CN	1,2-dioxoethyl	1,1-dimethylpropyl
818	1	bond	tetrazole	1,2-dioxoethyl	1,1-dimethylpropyl
819	2	bond	$CONH_2$	1,2-dioxoethyl	1,1-dimethylpropyl
820	1	bond	COOH	1,2-dioxoethyl	1,1-dimethylpropyl
821	2	bond	COOH	1,2-dioxoethyl	1,1-dimethylpropyl

Synthesis of Sensorineurotrophic Compounds